

**WHAT IS CLAIMED IS:**

1. A method for the structural analysis of endothelial nitric oxide synthase comprising:

- 5        a) subcloning a gene encoding the endothelial nitric oxide synthase in an expression vector;
- b) obtaining expression of the endothelial nitric oxide synthase protein from the vector;
- c) purifying the endothelial nitric oxide synthase protein;
- 10      d) preparing an endothelial nitric oxide synthase protein sample amenable for crystallization;
- e) crystallizing the endothelial nitric oxide synthase protein sample; and
- f) performing a three-dimensional structural analysis of the endothelial nitric oxide synthase by x-ray crystallography.

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2. The method of claim 1, wherein the endothelial nitric oxide synthase protein expressed is a variant endothelial nitric oxide synthase protein.

20      3. The method of claim 1, wherein the crystallizing comprises practicing a sitting drop vapor-diffusion method.

4. The method of claim 1, wherein the crystallizing is performed in the presence of tetrahydrobiopterin.

25      5. The method of claim 1, wherein the crystallizing is performed in the absence of tetrahydrobiopterin.

6. The method of claim 1, wherein the x-ray crystallography is performed in the presence of tetrahydrobiopterin.

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WO 00/37653

PCT/US99/30707

7. The method of claim 1, wherein the x-ray crystallography is performed in the absence of tetrahydrobiopterin.

8. A method of structural analysis to determine the binding of pterin to endothelial nitric oxide synthase comprising:

- a) determining the crystal structure of a dimeric heme domain of the endothelial nitric oxide synthase in the presence of the pterin; and
- b) determining the crystal structure of a dimeric heme domain of the endothelial nitric oxide synthase in the absence of the pterin;

and comparing the crystal structures.

9. The method of claim 8, wherein the pterin is tetrahydrobiopterin.

10. The method of claim 9, wherein the tetrahydobioppterin is (1'R,2'S,6R)-5,6,7,8-tetrahydrobiopterin.

11. The method of claim 8, wherein the crystal structure is determined at a resolution of between about 1.9 Å to about 2.1 Å.

20 12. A method for screening and identifying small molecule modulators of endothelial nitric oxide synthase proteins comprising:

- a) providing a pterin-free endothelial nitric oxide synthase structure;
- b) screening the small molecule modulators for their ability to bind to a pterin-binding site of the endothelial nitric oxide synthase ; and
- c) performing assays to determine the ability of the small molecule modulators to modulate the activity of endothelial nitric oxide synthase.

13. The method of claim 12, wherein the small molecule modulator inhibits endothelial nitric oxide synthase.

WO 00/37653

PCT/US99/30707

14. The method of claim 12, wherein the small molecule modulator activates endothelial nitric oxide synthase.

5 15. The method of claim 12, wherein the endothelial nitric oxide synthase protein expressed is a variant endothelial nitric oxide synthase protein.

16. The method of claim 12, wherein the pterin is tetrahydrobiopterin.

10 17. The method of claim 16, wherein the tetrahydrobiopterin is (1'R,2'S,6R)-5,6,7,8-tetrahydrobiopterin.

18. The method of claim 12, wherein the small molecule modulators are molecules and chemical-fragments from chemical-fragment libraries.

15 19. The method of claim 12, wherein the screening is performed by computerized methods.

20. The method of claim 12, wherein the assays are performed *in vitro* or *in vivo*.

21. A method for identifying drugs against diseased states in which nitric oxide signaling is defective or insufficient comprising:

- a) providing a tetrahydrobiopterin-free endothelial nitric oxide synthase structure;
- b) screening the drugs for their ability to bind the tetrahydrobiopterin binding site; and
- c) performing assays to determine the ability of the drugs to activate the endothelial nitric oxide synthase.

25 22. The method of claim 21, wherein the diseased states include impaired neurotransmission; impaired insulin release; impaired penile erection; impaired vasorelaxation; and impaired oxygen detection.

23. Endothelial nitric oxide synthase structure, obtained by the process comprising:

- a) subcloning a gene encoding the endothelial nitric oxide synthase in an expression vector;
- 5 b) obtaining expression of the endothelial nitric oxide synthase protein from the vector;
- c) purifying the endothelial nitric oxide synthase protein;
- d) preparing an endothelial nitric oxide synthase protein sample amenable for crystallization;
- 10 e) crystallizing the endothelial nitric oxide synthase protein sample.
- f) performing a three-dimensional structural analysis of the endothelial nitric oxide synthase by x-ray crystallography.

24. A nitric oxide synthase obtained according to the method of claim 1.

15 25. A method for screening and identifying a candidate substance with the ability to inhibit endothelial nitric oxide synthase comprising:

- a) obtaining a cell with endothelial nitric oxide synthase activity;
- b) admixing the candidate substance with the cell; and
- 20 c) determining the ability of the candidate substance to inhibit the endothelial nitric oxide synthase activity of the cell.

26. The method of claim 25, wherein the candidate substance is a small molecule modulator of endothelial nitric oxide synthase.

25 27. A method for screening and identifying a candidate substance with the ability to inhibit endothelial nitric oxide synthase comprising:

- a) obtaining a purified endothelial nitric oxide synthase;
- b) admixing the endothelial nitric oxide synthase with the candidate substance; and
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- c) performing X-ray crystallography analysis to determine the binding of the candidate substance.